experimentation. However, in some neuroendocrine cells, initial fusion pores may reopen several hundred times, indicating their stability. Moreover, these pores are too narrow to pass luminal molecules to the extracellular space, but their diameter can dilate upon stimulation. To explain the stability of the initial narrow fusion pores, anisotropic membrane constituents with non-axisymmetrical shape were proposed to accumulate in the fusion pore membrane. Although the nature of these is unclear, they may consist of lipids and proteins, including SNAREs, which may facilitate and regulate the pre- and post-fusional stages of exocytosis.

70-Subg
Dynamin-Catalyzed Membrane Fusion
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No abstract.

71-Subg
A Novel Player in Early Biogenesis of Insulin Granuals from Trans-Golgi Network
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No abstract.

72-Subg
Complexity of Complexin
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No abstract.

73-Subg
New Insights into the Molecular Mechanism of Calcium-Triggered Synaptic Vesicle Fusion
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The central nervous system relies on electrical signals travelling along neurons and through synapses at high speeds. Signals often have to pass between two neurons, or from a neuron to a muscle fiber, and the nervous system relies on a process called membrane fusion to ensure that the neurotransmitter molecules that carry the signal across the synapses are released as quickly as possible. Membrane fusion is an important process in many areas of biology, including intracellular transport and fertilization, but it occurs much faster (milliseconds) in the nervous system than anywhere else in the body. Moreover, it is precisely calcium regulated. The molecular mechanism that underlies this fast, regulated process has long been a mystery. We developed a single vesicle optical microscopy assay that directly monitors content-mixing (rather than just lipid-mixing) to observe membrane fusion between synthetic membranes that contain reconstituted synaptic proteins: neuronal SNAREs, synaptotagmin, complexin, and Munc18. When calcium ions were injected into our synthetic system, the basic characteristics of neurotransmitter release - such as membrane fusion on a millisecond time scale - was observed. Remarkably, the fastest fusion events did not begin or proceed via a discernible hemifusion intermediate state. Rather, these events proceeded from a ''point contact'' state in which the membranes were close to each other without being fused, and were ready to undergo fast fusion once the calcium ions had been injected. When we introduced a protein called complexin, which is known to be important for fast neurotransmitter release in vivo, we observed more immediate fusion events.
References
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Subgroup: Permeation & Transport

74-Subg
Ion Conduction Mechanism of a Viral Proton Channel from Solid-State NMR
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The M2 protein of the influenza virus forms a proton-selective channel that is important for the lifecycle of the virus. Using high-resolution magic-angle-spinning (MAS) solid-state NMR spectroscopy, we have obtained rich information on the proton-conduction mechanism in this prototypical ion channel. The proton-selective residue, a histidine in the transmembrane (TM) domain of M2, undergoes microsecond reorientations, at the same time exchanging protons with water molecules. The rate and energy barrier of the reorientation motion and the kinetics of proton exchange are quantified through measurements of ^{15}N exchange NMR spectra, motionally averaged nuclear-spin couplings, and ^{1}H chemical shifts. The pH-dependent charged state and the rotameric structure of the histidine rings were determined from chemical shifts and internuclear distances. Aromatic interactions between the histidine and an adjacent tryptophan residue provide novel insight into the two-orders-of-magnitude difference between the histidine-water proton exchange rate and the actual proton flux into the virion. Comparison of the ^{15}N spectra of wild-type M2 with a mutant with higher proton conductance revealed that histidine - water proton exchange not only involved transformation of the neutral imidazole to the cationic imidazolium, but also involved transitions between the two neutral tautomeric states of histidine, indicating that quantum-mechanical tunneling is a second mechanism for proton relay. Mechanistic insights obtained from the TM domain of M2 are extended to influenza B virus M2 and to a longer construct of the protein to investigate the influence the extra-membrane domains on proton conduction.
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75-Subg
All-Atom Simulation of ION Permeation in Single-File Channels
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Using long, all-atom simulations enabled by special-purpose hardware, we studied K^+ permeation at the level of individual permeation events across the bacterial channel gramicidin A and across the $K_V1.2/2.1$ voltage-gated potassium channel. At experimentally accessible voltages, which include the physiological range, the simulated permeation rates were substantially lower than the experimentally observed rates for both systems. In addition, the current-voltage relationships were nonlinear, but became linear at much higher, non-physiological voltages. In gramicidin A, the underestimated permeation rate largely resulted from too-infrequent ion recruitment into the pore lumen, although reducing the interaction strength between the ion and the pore did increase the observed permeation rate. In $K_V1.2/2.1$, we observed at all voltages permeation consistent with a knock-on mechanism, and found that the predicted rate was lower than the experimental rate because the knock-on intermediate formed too infrequently. Additional simulations further suggested that the knock-on permeation mechanism in different K^+ channels could vary, possibly due to sequence and structural variations in the selectivity-filter regions of these channels. Despite the need to apply large voltages to simulate the permeation processes, the apparent voltage insensitivity of the permeation mechanisms overall suggests that the direct simulation of permeation at the single-ion level can provide fundamental physiological insight into ion channel function. Polarizable force fields and membrane models with improved dipolar potential and dielectric constants would be needed, however, to obtain accurate simulated permeation rates at experimentally accessible voltages.